NISTTech

REFERENCE STAIN, PROCESS FOR STAINING AND ACQUIRING NORMALIZED SIGNAL

NIST Docket 13-020

Applications

- **Medicine**
  Can be used for medical and biological research
- **Cell based products**
  Companies that produce cell based products

Advantages

- **No reference material**
  No reference material is needed for this method
- **Easier applications**
  Some of the techniques in the present invention facilitate comparison of either biomarker intensity, or concentration after normalization to protein density

Abstract

There is great need for strategies that enable comparability of antibody-based measurements within and between flow and imaging cytometry. Fundamental to this task are reference materials that normalize fluorescence measurements from one instrument to another. We are attempting to use reactive fluorescent dyes that reproducily and non-specifically label total cellular proteins as benchmarks for antibody-binding studies. Our work shows that commerically available maleimide dyes, which covalently bind to free cytesine grouos in permeabilized cells and are correlated with protein concentration (Pretzer and Wiktorowicz; Anal Biochem; 2008), can serve as an internal fluorescence reference for fluorescent antibody-based measurements. To evaluate the reproducibility of these benchmark measurements, the robustness of the maleimide staining was tested. We determined that maleimide labeling could be saturated by controlling maleimide concentration and labeling time. We found the protocol was most robust to these parameters when stained for more than 30 min with greater than 10 ug/ml maleimide concentration. Immediately prior to labeling, we also determined that cells should be fixed a minimum of 3 hours in paraformaldehyde, which reduces disulfids bonds, in order to acheive maximum labeling. The ability to reproducily achieve maximum saturation labeling enables the malemide of fluorescent antibodies or biomarkers. As proof of principle, we demonstrated that cells co-stained with Alexa488-labeled phalloidin and Alexa 647-maleimide could benormalized across 8 different filte3r, lamp and camera settings covering 13 lab-to-lab comparably between antibody based measurements in both flow and imaging cytometry.

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Status of Availability

This invention is available for licensing exclusively or non-exclusively in any field of use.

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